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Seasonal variations in eelgrass (*Zostera marina* L.) responses to nutrient enrichment and reduced light availability in experimental ecosystems

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Abstract

The single and interactive effects of altered water column nutrient concentrations and light availability on the growth of the seagrass *Zostera marina* L. (eelgrass) and its attached epiphytes were investigated in 110 liter microcosms. Experiments lasting 4 to 6 weeks were conducted seasonally during spring, summer and fall in a greenhouse equipped with flow-through seawater from the adjacent York River estuary of the Chesapeake Bay. Nutrient treatments consisted of inflow seawater with ambient or enriched ($2\times$ to $3\times$) concentrations of dissolved inorganic nitrogen and phosphorus and with rapid turnover (16 d^{-1}). Enrichment levels were chosen to evaluate conditions found in regions of the Chesapeake Bay where *Z. marina* has declined. Light reductions were accomplished by shading individual microcosms with neutral density screening so that mean scalar irradiance was 42, 28, or 9% of solar PAR. These levels were chosen to simulate light reductions observed along gradients of turbidity which characterize present and former *Z. marina* habitats in the region. Epiphytic grazers consisted of gastropods (*Bittium varium* and *Mitrella lunata*) which were applied at consistent densities (5200 m^{-2}) for all experiments. Growth of both the seagrasses and their associated epiphytes decreased with increased shading. There was little additional response to nutrient enrichment except at highest light levels during the spring when macroepiphytes increased to over $10\times$ the seagrass mass and seagrass growth decreased. The results suggest that it is principally light availability which governs seagrass growth in moderately nutrient enriched regions of the bay. In systems such as the York River, given adequate grazer densities, observed levels of nutrient enrichment are unlikely to cause excessive epiphyte loads and subsequent seagrass declines. Although *Z. marina* tissue levels of nitrogen and phosphorus increased significantly with enrichment and with shading no direct effects of nitrate toxicity were observed.

Keywords: *Zostera marina* L; Eelgrass; Experimental ecosystems; Light availability; Epiphytes; Nutrients

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1. Introduction

The declines of seagrass (e.g., *Zostera marina* L., eelgrass) and other submersed vascular plant communities worldwide have been attributed to deteriorating habitat conditions (den Hartog and Polderman, 1975; Orth and Moore, 1983; Silberstein et al., 1986). Nutrient enrichment can promote phytoplankton growth (Phillips et al., 1978; Boynton et al., 1982; Sand-Jensen and Borum, 1991) which increases turbidity (Dennison et al., 1989), and runoff of suspended sediments and dissolved substances further increases water column light attenuation, especially in estuarine areas (Champ et al., 1980; Kemp et al., 1983; McPherson and Miller, 1987; Moore et al., 1997). Decreased light availability can have adverse effects on *Z. marina* photosynthesis (Dennison and Alberte, 1982), growth (Dennison and Alberte, 1985; Dennison, 1987), community structure (Backman and Barilotti, 1976; Short et al., 1995), and ultimately long-term survival (Zimmerman et al., 1995; Moore et al., 1996). With increased nutrient loading, seagrasses have been replaced by macroepiphytes in some systems (Duarte, 1995; Coffaro and Bocci, 1997; Valiela et al., 1997). Nutrient enrichment has also been related to increased epiphytic growth on macrophyte shoots in the field (Cambridge and McComb, 1984; Borum, 1985). Epiphytes are thought to reduce plant growth by reducing light availability as well as limiting bicarbonate uptake for photosynthesis (Sand-Jensen, 1977). Additionally, there is some evidence that elevated concentrations of water column nitrate may be toxic to some submersed macrophytes (Burkholder et al., 1992, 1994).

Survival of submersed plants is dependent on the interactions of many biotic and abiotic factors that affect the balance between production and respiration (Zimmerman et al., 1995). Little is known, however, about the combined influences of many of these factors that operate simultaneously in nature. For example, in many areas, such as the York River in Virginia where seagrasses have declined, both water column turbidity and nutrients are greater in impacted sites than unimpacted sites (Batuik et al., 1992; Moore et al., 1996). Although such field observations can be used to infer the possible linkages between habitat conditions and macrophyte survival (Dennison et al., 1993), they provide only correlative information. Controlled experiments are necessary to define the causal relationships between multiple controlling factors and seagrass growth (e.g., Barko et al., 1991; Neckles et al., 1993).

Many nutrient enrichment studies on macrophytes attempt to simulate nutrient loadings to a system using pulsed inputs. Usually water turnover is low and initial levels of enrichment are very high, ranging up to 100× of controls, followed by rapid decreases in water column concentrations with time (Burkholder et al., 1992; Neundorfer and Kemp, 1993). However, field observations suggest that rapid turnover of water combined with constant but lower increases in water column nutrient concentrations (e.g., 2× to 3×; Neckles et al., 1993) are more characteristic of differences between many regions where *Z. marina* has survived and areas where it has died out (Batuik et al., 1992; Moore et al., 1996).

In addition, while experiments have demonstrated that long-term light reductions can significantly impact *Z. marina* communities (Backman and Barilotti, 1976; Short et al., 1995), in natural systems relatively short-term periods of high turbidity may be limiting

Z. marina survival (Zimmerman et al., 1995) and seasonal reductions of 30 to 40 days in duration are sufficient to limit *Z. marina* survival (Dennison and Alberte, 1985; Moore et al., 1997).

In this study, the seasonal responses of *Z. marina* and its epiphyte community to decreased water column light availability and increased nutrient concentrations under conditions of rapid water turnover were investigated. The objective was to determine the relative importance of these two environmental factors which have been related to *Z. marina* declines and to determine their single and interactive effects on *Z. marina* communities in experimental microcosms.

2. Methods

2.1. Experimental design

The effects of light reduction and dissolved inorganic nitrogen and phosphorus enrichment on field-collected *Z. marina* and its epiphytes were determined in three, seasonally replicated microcosm experiments. Experiments were timed to correspond to temperature-based seasonal patterns of *Z. marina* growth in the Chesapeake Bay (Orth and Moore, 1986; Moore et al., 1996). Both the spring and fall represented periods of high *Z. marina* growth, while the summer represented a period of low growth. A randomized complete block, 3 (light) $\times$ 2 (nutrient) factorial design with four replicates was applied to the microcosms in each experiment. The experimental system consisted of 24, 110 liter glass aquaria located in a greenhouse supplied with continuously pumped and filtered seawater from the adjacent York River estuary, Chesapeake Bay (37°15′02″N, 76°29′44″W). Blocks consisted of adjacent, paired seawater tables to which each of the six treatments were randomly assigned, three per table (Fig. 1). Each table was individually supplied with seawater which was sand filtered, then 30 μm bag filtered into a header tank which supplied water at a constant rate with a turnover time of 16 d^{-1}. Conditions were established to simulate the in situ environment of *Z. marina* within the lower Chesapeake Bay region. Salinities were ambient and daily average water temperatures in the microcosms were within 1°C of ambient York River temperatures. Light conditions were natural sunlight reduced only by the greenhouse and neutral density covers (Chicopee, Inc.) which surrounded individual aquaria. Water turnover rates were at high levels to simulate conditions of high water exchange common in seagrass beds in the lower Chesapeake Bay region. Nutrient treatments in each of the four replicate blocks were individually supplied with continuous nutrient amendments from separate reservoirs by peristaltic pump (Masterflex®, Cole-Palmer Instrument Co.) which was mixed with the inflow water prior to entering a microcosm. Each microcosm was aerated continuously and water was additionally circulated during daylight hours with submersed pumps and PVC diffusers to minimize carbon limitation and formation of boundary layers at the leaf surfaces. Current velocities in the microcosms were approximately 2–9 cm s^{-1}, which are within the range of those

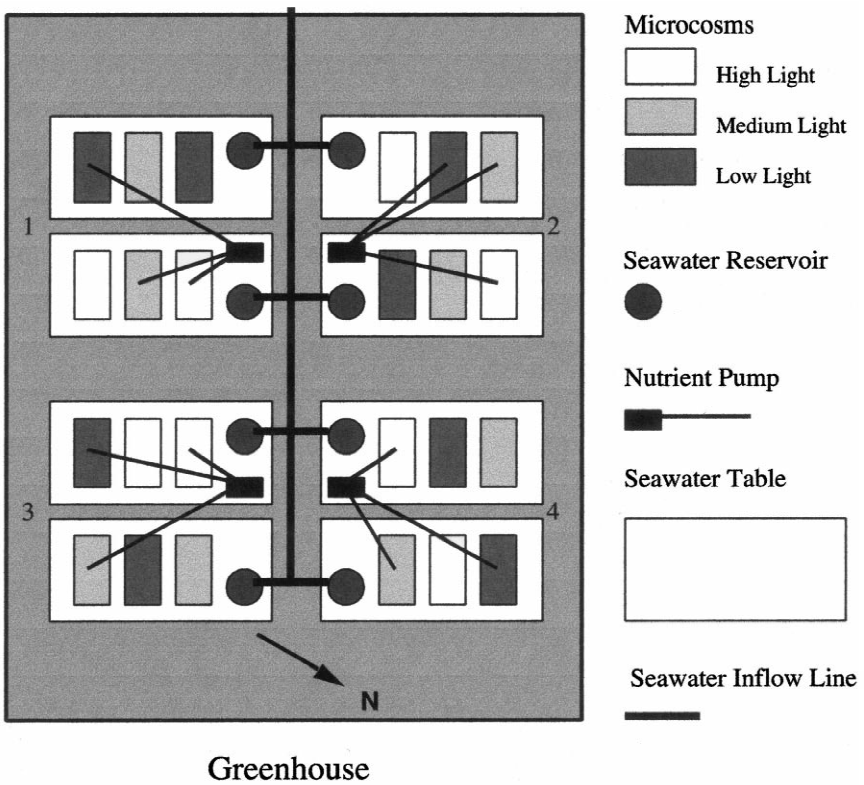

Fig. 1. Typical design for flow-through seawater greenhouse experiments. A block consisted of paired tables of six experimental chamber treatments with three enriched by nutrients.

reported for natural *Z. marina* communities (Harlin and Thorne-Miller, 1981; Fonseca et al., 1983; Seufzer, 1994).

Grazer densities within all treatments and experiments were held constant and numbers of grazing gastropods (*Bittium varium* and *Mitrella lunata*) were based upon field epifauna densities associated with *Z. marina* communities in the lower bay (Marsh, 1973; Neckles, 1990). Tanks were regularly checked for grazer numbers by arbitrarily removing pots and counting the gastropods. Occasionally organisms were added, if needed, throughout the course of the experiments. If additional grazer recruits such as small amphipods or copepods were observed in the chambers, the microcosms were flushed with freshwater for 5–10 min (cf. Duffy, 1990) and gastropods restocked as necessary.

All microcosms were cleaned approximately twice weekly to remove epiphytic growth from the walls, and debris was siphoned from the bottom. All material was screened and any gastropods were returned. High water turnover rates helped maintain low phytoplankton concentrations, which were checked periodically from randomly

selected microcosms in each treatment. Water samples were filtered using Whatman GF/F glass fiber filters and chlorophyll extracted in a solvent mixture of acetone, dimethyl sulfoxide and 1% diethylamine (45:45:10, v/v) and determined fluorometrically (Shoaf and Lium, 1976). Mean chlorophyll *a* concentrations (5–20 $\mu g\ l^{-1}$) approximated field concentrations (Batuik et al., 1992; Moore et al., 1996) and did not differ significantly among treatments.

Physical conditions, including light and temperature, were measured continuously and recorded as mean (temperature) or integrated (light) at 15 min intervals. Photosynthetically active radiation (PAR) was measured in the water at mid-canopy height in one, unshaded tank, after preliminary measurements indicated little measurable difference ($< 5\%$) in total irradiance over 24 h periods with aquarium location in the greenhouse. Microcosm PAR are reported as scalar irradiance (LI-COR, Inc., Model LI-193 SA). Atmospheric PAR, measured as downwelling irradiance (Li-COR, Inc., Model LI-190 SA), was recorded simultaneously, outside the greenhouse at the Gloucester Point, Va. (37°15′02″N, 76°29′44″W) research site. Water temperatures were recorded by thermistor. Diel variation in water temperatures averaged less than 3°C and discreet measurements among individual aquaria showed little variation (< 2°C).

Whole plants, including roots and rhizomes, were collected from a historically stable *Z. marina* bed at the mouth of the York River (37°16′44″N, long. 76°20′44″W) approximately 2 to 3 weeks prior to the initiation of each experiment. The plants were washed free of sediments and transported to the greenhouse for replanting in 11.4 cm plastic pots filled with 5 mm sieved and homogenized sediments collected from the field site. Individual shoots selected for replanting were standardized by selecting non-reproductive shoots having at least four leaves with rhizomes cut distal to the fifth internode. Shoots were planted at average field densities (1500 m^{-2}) observed previously for the lower York River (Orth and Moore, 1986), and placed in running, 30 μm filtered seawater tanks in the greenhouse for acclimatization (ca. 2–3 weeks) under high light–ambient nutrient treatment conditions until initiation of the experiment.

Immediately prior to the start of each experiment a 2×5 cm deep sediment core was obtained from each of three pots. Nutrients were extracted in 1 N KCl for 25 min and centrifuged. Concentrations of ammonium (NH_4^+) were determined immediately (Solorzano, 1969). Samples were frozen for later analyses of nitrite + nitrate ($NO_2^- + NO_3^-$), and dissolved inorganic phosphate (PO_4^{3-}) using standard colorometric techniques (Parsons et al., 1984) or a Technicon Autoanalyzer.

2.2. Treatment applications

Light levels were chosen to examine the effects of light reductions observed along a gradient of turbidity which characterizes *Z. marina* habitats in the lower Chesapeake Bay and its tributaries. The low light level was chosen to simulate light levels which over the short term (30 days at 3–4 mol d^{-1}; 9% incident solar PAR) have been related to dieoffs of *Z. marina* both in the Chesapeake Bay and elsewhere (Dennison and Alberte, 1985; Olesen and Sand-Jensen, 1993; Moore et al., 1997). High light levels (42% incident solar PAR) were chosen to approximate light levels regularly measured at historically stable *Z. marina* beds (Dennison and Alberte, 1985; Moore et al., 1996),

while medium light levels (28% incident solar PAR) approximated sites of transitional light intensity characteristic of ephemeral seagrass beds for this region (Batuik et al., 1992; Moore et al., 1997).

Nutrient levels were chosen to evaluate the effects of constant enrichment in water column nutrient concentrations over different seasons. High turnover rates (1.5 h) were used to simulate rapid exchange of water through seagrass beds. Enrichment levels were characteristic of nutrient concentrations in formerly vegetated sites in the York River that have been correlated to *Z. marina* decline in this region (Neckles et al., 1993; Moore et al., 1996). Ambient levels were characteristic of sites in the York River where *Z. marina* has remained vegetated historically. Dissolved inorganic nitrogen (DIN) concentrations were measured as the sum of nitrite and nitrate (NO_x^-), and ammonium (NH_4^+). Dissolved inorganic phosphate (DIP) was measured as orthophosphate (PO_4^{3-}). Concentrations were determined spectrophotometrically (NO_x^- and PO_4^{3-}, USEPA, 1979; NH_4^+, Parsons et al., 1984) at weekly intervals directly from the inflow and outflow of each of the microcosms. Enrichments of DIN and DIP ranged from $2\times$ to $3\times$ during the course of the experiments while DIN/DIP ratios remained consistent between ambient and enriched treatments.

2.3. Macrophyte growth response

At the start of each experiment six numbered pots were randomly assigned to each microcosm. Every 2 weeks one pot was randomly selected from each microcosm for macrophyte mass and growth measurements. Growth during the 2 week sampling intervals was measured using a modification of the leaf marking technique of Sand-Jensen (1975). All plants in the selected pots were marked with small notches in each leaf approximately 2–3 cm above the sediment surface at the start of the growth interval, and growth was measured as the length and width of all leaf material produced during the interval. At the end of each growth interval the selected pots were gently rinsed, cleaned of epiphytes and separated into leaves and root–rhizome components. Linear regressions of dry weight versus leaf area were derived from leaves processed for epiphyte loads (see below) from companion pots and used to calculate shoot mass ($R^2 > 0.96$). Shoot and root–rhizome mass was calculated as the weight of each component measured at the end of the growth intervals. Shoot growth was calculated both as new leaf mass divided by the initial number of shoots and the pot area. Specific leaf growth was calculated as new leaf mass divided by the initial mass. Growth and production measures are presented as daily rates. The plastochrome interval (PI), or the average period in days for the emergence of a new leaf, was calculated as the inverse of the number of new leaves produced per shoot over the growth interval.

Subsamples of shoot and root–rhizome tissue were freeze dried, ground in a Wiley mill and analyzed in duplicate for total carbon and nitrogen (Perkin-Elmer, Model 501B, CHN analyzer). Subsamples were analyzed colorimetrically for P content after oxidation following Solorzano and Sharp (1980) as modified by Fourqurean and Zieman (1992).

2.4. Epiphyte response

Epiphyte mass was measured as weight on plants in one randomly selected pot per

microcosm at each biweekly sampling date. Epiphytes were scraped from leaves with the edge of a glass slide into filtered seawater. Macroepiphytes were separated by rinsing the scraped material through a sieve (80 mesh). The remaining microepiphytes were collected by filtration onto precombusted and preweighed Gelman A/E glass fiber filters. The filters were further rinsed with deionized water to remove seasalts. The macroepiphytes were similarly rinsed a second time and placed on precombusted and preweighed aluminum pans. Epiphyte dry mass was determined after drying at 60°C for 2–5 days and ash-free dry mass after combustion at 500°C for 5 h. *Z. marina* leaf area was measured using an area meter (LI-COR, Inc., Model 3100) and ash-free and dry shoot mass determined as for the epiphytes.

During the summer experiment artificial eelgrass substrates were used to measure epiphyte accrual rates. Each unit of substrate consisted of four strips of polypropylene ribbon (0.5 cm×35 cm) attached to mats of plastic mesh. The artificial substrates were placed in the microcosms and additional grazers were added at densities equivalent to that of the natural eelgrass substrate, assuming one-to-one equivalency of artificial to natural eelgrass shoot surface area. At weekly intervals 20 artificial substrates with the appropriate number of grazers were removed from each microcosm. Epiphytes were scraped from the artificial substrates and processed for microepiphytes and macroepiphytes as described for the natural shoots.

2.5. Statistical analyses

All responses to treatments within each experiment were tested using repeated measures analysis of variance with between subjects main effects of nutrient level, light level and between subjects main effects of time. Dependent variables were log-transformed, when indicated by residual analysis (Neter and Wasserman, 1974), to produce homogenous variance. Factor level means were compared post hoc using Tukey's HSD (Neter and Wasserman, 1974). All analyses were performed using the MANOVA procedure of STATISTICA/MAC, StatSoft, Inc.

3. Results

3.1. Experimental conditions

Environmental conditions in the microcosms during the three experiments (Table 1) reflected seasonal patterns in the natural environment and represented the range of conditions observed for *Z. marina* vegetated and unvegetated habitats in the York River estuary (Batuik et al., 1992; Moore et al., 1996). Average daily solar irradiance was similar during the spring and summer experiments and lowest during the fall. Water temperature range was similar during the spring and fall experiments: increasing (12 to 19°C) throughout the spring and decreasing (17 to 12°C) throughout the fall. Summer water temperatures increased to a maximum of 30°C by the end of the experiment. Salinities reflected conditions in the York River source water and were similar in the summer and fall, and slightly lower in the spring.

Inflow nutrient concentrations ranged from 5 to 20 μM for DIN and 0.2 to 2.0 μM for

Table 1
Experimental conditions during seasonal microcosm studies

	Summer	Fall	Spring
Date (mm/dd)	7/8–8/15	11/7–12/8	4/5–5/18
Solar PAR (mol m^{-2} d^{-1})	37	20	34
Light transmission (mol m^{-2} d^{-1}; % solar PAR)			
High light	15.5 (42)	8.4 (42)	14.3 (42)
Medium light	10.4 (28)	5.6 (28)	9.5 (28)
Low light	3.3 (9)	1.8 (9)	3.1 (9)
Water temperature (°C)	24–29	17–12	12–19
DIN (μM; μmol l^{-1} d^{-1}) inflow			
Ambient treatments	6.3 (101)	8.9 (142)	6.9 (110)
Enriched treatments	14.6 (234)	17.2 (275)	16.9 (270)
NO_x^- (μM; μmol l^{-1} d^{-1}) inflow			
Ambient treatments	3.8 (61)	4.5 (72)	3.1 (50)
Enriched treatments	7.6 (122)	7.7 (275)	6.6 (106)
NH_4^+ (μM; μmol l^{-1} d^{-1}) inflow			
Ambient treatments	2.5 (40)	4.4 (70)	3.8 (61)
Enriched treatments	7.0 (112)	7.7 (123)	10.3 (165)
PO_4^{3-} (μM; μmol l^{-1} d^{-1}) inflow			
Ambient treatments	0.8 (13)	0.9 (14)	0.5 (8)
Enriched treatments	1.7 (27)	1.8 (29)	1.4 (22)
Sediment porewater (μM)			
NO_x^-	10	15	5
NH_4^+	70	65	60
PO_4^{3-}	5	10	10
Chl a (μg l^{-1}) outflow	5–20	5–20	5–20
Salinity (PSU)	22–23	20–23	16–19
Grazers (m^{-2})			
Bittium varium	5000	5000	5000
Mitrella lunata	200	200	200
Z. marina density (m^{-2})	1500	1500	1500

DIP with highest concentrations during the fall and lowest during the summer (Table 1). Enrichment increased DIN and DIP approximately 10 μM and 1 μM, respectively, or from 2× to 3× ambient levels. DIN dosages ranged from 101 to 142 and 234 to 275 μmol N per liter of microcosm volume per day for ambient and enriched treatments respectively (Table 1). DIP dosages similarly ranged from 8 to 13 and 22 to 29 μmol P per liter of chamber volume per day. DIN/DIP ratios of approximately 10:1 observed in the inflow were maintained with enrichment, as were the nearly equal proportions of NO_x^- and NH_4^+ of the inflow water. Initial sediment nutrient concentrations, reflecting the common source of material, were consistent among experiments with NH_4^+ comprising the majority of extractable DIN (Table 1).

3.2. Z. Marina response

Seagrass tissue carbon content demonstrated no significant changes ($P > 0.05$) either over the course of each experiment, or in response to light reductions or nutrient enrichments (Table 2). Both shoot and root–rhizome levels (Table 3) were lower by the end of the summer experiments (35.5 to 37.5% and 26.6 to 29.2%, respectively) than during the fall (36.5 to 39.2% and 26.6 to 31.4%) and highest during the spring (37.5 to 39.0% and 33.1 to 33.7%).

The eelgrass shoots demonstrated significantly decreased ($P < 0.05$) nitrogen content with increasing light level during all three seasons (see Table 2; data not shown). Levels were also significantly higher in the enriched treatments at each light level during the summer and spring, but no response to enrichment level was observed during the fall when tissue nitrogen content was greatest. Nitrogen content generally increased with time (Table 2; data not shown) with maximum response to treatment observed by the end of each experiment (Table 3). In contrast to the shoots, root–rhizome tissue nitrogen

Table 2
Analysis of variance of *Zostera marina* tissue carbon, nitrogen, phosphorus responses with repeated measurements on microcosms. N, nutrient; L, light; T, time effects; R–R, root–rhizome. Bold indicates significant effect ($P < 0.05$)

Treatment	DF	Shoot carbon		Shoot nitrogen		Shoot phosphorus		R–R carbon		R–R nitrogen		R–R phosphorus	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Summer													
N	1	0.66	0.440	28.19	**0.001**	11.02	**0.004**	0.08	0.788	0.04	0.855	0.84	0.373
L	2	0.51	0.614	11.96	**0.004**	1.05	0.373	0.07	0.934	1.99	0.207	0.36	0.703
N×L	2	1.13	0.367	0.64	0.551	0.60	0.561	0.12	0.882	1.35	0.318	1.60	0.212
T	2	2.50	0.113	29.27	**0.000**	5.53	**0.009**	0.29	0.753	15.89	**0.000**	3.86	0.032
N×T	2	1.30	0.299	0.79	0.469	0.08	0.925	1.37	0.286	5.14	**0.021**	0.91	0.415
L×T	4	1.06	0.410	3.27	**0.039**	0.41	0.801	0.61	0.660	1.48	0.261	0.88	0.498
N×L×T	4	1.24	0.333	0.11	0.978	0.37	0.825	0.37	0.825	0.99	0.444	1.57	0.207
Fall													
N	1	0.00	0.999	0.66	0.430	0.07	0.802	0.45	0.512	8.14	**0.012**	4.12	0.057
L	2	2.42	0.116	5.02	**0.021**	0.23	0.798	0.27	0.768	3.39	0.609	1.15	0.340
N×L	2	0.09	0.916	3.28	0.066	0.02	0.985	2.04	0.100	4.20	**0.036**	0.17	**0.000**
T	2	2.22	0.157	18.49	**0.001**	52.34	**0.000**	0.74	0.402	6.12	**0.026**	18.25	**0.000**
N×T	2	2.38	0.144	4.33	0.055	1.14	0.300	0.08	0.788	0.25	0.624	0.01	0.925
L×T	4	0.00	0.992	0.57	0.578	9.50	**0.002**	2.59	0.109	0.13	0.882	0.12	0.890
N×L×T	4	0.84	0.452	1.02	0.383	0.39	0.680	2.05	0.163	0.80	0.469	0.46	0.640
Spring													
N	1	1.39	0.253	23.00	**0.000**	7.51	**0.013**	0.91	0.383	17.26	**0.009**	0.81	0.404
L	2	0.56	0.581	11.14	**0.001**	0.85	0.280	2.08	0.221	4.76	0.070	1.68	0.290
N×L	2	0.18	0.836	0.81	0.460	2.52	0.109	0.76	0.514	2.07	0.222	1.24	0.355
T	2	3.79	0.067	0.01	0.910	5.80	**0.027**	0.83	0.463	11.07	**0.003**	8.31	**0.005**
N×T	2	1.07	0.316	0.09	0.769	0.00	1.000	0.13	0.880	0.97	0.411	0.45	0.650
L×T	4	0.21	0.809	0.43	0.660	4.89	**0.020**	1.00	0.451	1.14	0.392	0.51	0.731
N×L×T	4	0.66	0.528	1.97	0.168	0.35	0.711	0.16	0.954	1.59	0.252	0.58	0.682

Table 3
Mean (SE) *Z. marina* tissue carbon (C), nitrogen (N) and phosphorus (P) content at the end of each seasonal study

Season	Treatment		Shoot			Root–rhizome		
	Nutrients	Light	% C	% N	% P	% C	% N	% P
Summer	Ambient	High	35.5 (0.9)	2.0 (0.1)	0.22 (0.00)	28.3 (2.1)	1.1 (0.0)	0.07 (0.01)
	Ambient	Medium	37.5 (0.2)	2.2 (0.1)	0.22 (0.01)	29.0 (1.9)	1.2 (0.0)	0.07 (0.01)
	Ambient	Low	37.1 (0.3)	2.5 (0.0)	0.23 (0.01)	29.2 (1.1)	1.3 (0.1)	0.06 (0.00)
	Enriched	High	36.5 (1.0)	2.4 (0.0)	0.25 (0.00)	27.0 (2.3)	1.2 (0.0)	0.07 (0.00)
	Enriched	Medium	36.8 (0.5)	2.5 (0.0)	0.24 (0.01)	27.2 (1.5)	1.2 (0.1)	0.07 (0.00)
	Enriched	Low	36.9 (0.8)	3.0 (0.1)	0.25 (0.02)	26.6 (2.1)	1.3 (0.1)	0.07 (0.01)
Fall	Ambient	High	37.8 (1.2)	3.5 (0.1)	0.25 (0.01)	26.6 (2.3)	1.4 (0.1)	0.09 (0.01)
	Ambient	Medium	38.0 (1.5)	3.8 (0.2)	0.26 (0.01)	30.4 (1.6)	1.6 (0.0)	0.08 (0.01)
	Ambient	Low	39.2 (0.5)	3.8 (0.1)	0.22 (0.01)	31.0 (0.7)	1.4 (0.1)	0.07 (0.01)
	Enriched	High	38.1 (0.3)	3.6 (0.0)	0.26 (0.01)	30.7 (0.6)	1.9 (0.1)	0.10 (0.01)
	Enriched	Medium	36.5 (1.9)	3.6 (0.1)	0.26 (0.02)	30.5 (1.6)	1.9 (0.2)	0.09 (0.00)
	Enriched	Low	38.0 (0.7)	3.6 (0.1)	0.24 (0.01)	31.4 (0.9)	1.4 (0.1)	0.09 (0.01)
Spring	Ambient	High	37.6 (0.3)	2.0 (0.1)	0.21 (0.01)	34.2 (1.0)	1.2 (0.0)	0.11 (0.01)
	Ambient	Medium	38.1 (0.9)	2.8 (0.5)	0.19 (0.01)	33.2 (1.8)	1.1 (0.1)	0.12 (0.01)
	Ambient	Low	37.5 (0.3)	2.7 (0.0)	0.20 (0.02)	34.7 (0.1)	1.7 (0.1)	0.12 (0.01)
	Enriched	High	39.0 (1.7)	2.8 (0.1)	0.23 (0.01)	34.2 (0.5)	1.8 (0.2)	0.14 (0.01)
	Enriched	Medium	38.9 (0.7)	2.9 (0.1)	0.21 (0.01)	33.1 (1.2)	1.8 (0.2)	0.13 (0.02)
	Enriched	Low	38.0 (0.3)	3.2 (0.1)	0.23 (0.01)	33.6 (1.5)	2.0 (0.2)	0.15 (0.02)

demonstrated no significant effect of light or nutrient level during the summer ($P > 0.10$; Table 2). However, root–rhizome nitrogen content was significantly higher with enrichment at all three light levels during the spring and the highest two light levels during the fall ($P < 0.05$; Table 3). Highest seasonal seagrass tissue nitrogen levels at this time (3.5 to 3.8%; Table 3) suggest nitrogen was potentially less limiting in the fall than during the spring (2.0 to 3.2%) and summer (2.0 to 3.0%). This was also the period when overall N and P inflow concentrations were highest and incident light the lowest. Shoot tissue P levels generally paralleled tissue nitrogen and were significantly higher in enriched treatments during the summer and spring ($P < 0.05$; Tables 2 and 3), however no effect of light level was observed ($P > 0.05$). Root–rhizome P content demonstrated no consistent response to light or nutrient treatments, however levels were seasonally highest at the end of the spring experiment (0.11 to 0.15%; Table 3).

Z. marina growth demonstrated significant changes over the course of each experiment, reflecting not only the normal seasonal patterns observed in field populations (Orth and Moore, 1983) but also the compounding treatment effects of light availability, nutrient enrichments and the indirect effects of epiphyte loading (Table 4; Figs. 2 and 3). Interaction effects were generally not significant except during the spring (Table 4), therefore the effects of light and time were additive during the summer and fall. The four measures of shoot production presented here can vary independently with treatment

Table 4
Analysis of variance of *Zostera marina* growth responses with repeated measurements on microcosms. N, nutrient effect; L, light effect; T, time effect. Bold indicates significant effect ($P < 0.05$)

Treatment	DF	Shoot biomass production (g m^{-2} d^{-1})		Shoot growth (mg $shoot^{-1}$ d^{-1})		Shoot growth (% d^{-1})		Plastochrome interval (d)	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Summer									
N	1	3.91	0.053	6.58	0.130	4.71	**0.035**	0.20	0.089
L	2	5.98	**0.005**	5.94	**0.005**	11.51	**0.000**	3.12	0.053
N×L	2	1.10	0.339	0.81	0.449	0.47	0.625	0.25	0.776
T	2	106.82	**0.000**	86.27	**0.000**	30.51	**0.000**	45.22	**0.000**
N×T	2	1.25	0.294	1.02	0.366	0.99	0.378	2.65	0.080
L×T	4	0.28	0.891	0.42	0.795	1.52	0.209	1.05	0.392
N×L×T	4	3.06	0.024	1.38	0.254	0.75	0.563	0.41	0.800
Fall									
N	1	0.95	0.336	2.07	0.160	1.43	0.240	0.03	0.865
L	2	64.55	**0.000**	55.22	**0.000**	71.88	**0.000**	9.21	**0.001**
N×L	2	1.34	0.277	1.09	0.349	1.67	0.204	0.19	0.981
T	2	106.91	**0.000**	170.28	**0.000**	81.62	**0.000**	9.91	**0.004**
N×T	2	0.61	0.439	0.40	0.534	0.21	0.654	0.08	0.774
L×T	4	0.38	0.686	1.15	0.330	0.27	0.763	1.77	0.187
N×L×T	4	0.74	0.484	0.05	0.950	0.81	0.835	0.07	0.927
Spring									
N	1	5.37	**0.025**	5.66	**0.020**	3.89	**0.048**	8.73	**0.006**
L	2	6.43	**0.004**	6.12	**0.004**	7.76	**0.002**	11.45	**0.000**
N×L	2	0.29	0.746	0.24	0.788	2.31	0.115	0.37	0.690
T	2	6.69	**0.014**	5.54	**0.025**	2.76	0.106	10.62	**0.003**
N×T	2	0.11	0.745	0.01	0.925	0.36	0.555	0.80	0.376
L×T	4	4.05	**0.027**	3.61	**0.038**	1.99	0.152	4.16	**0.024**
N×L×T	4	0.48	0.818	0.98	0.385	0.20	0.819	1.13	**0.036**

(e.g., Short et al., 1995). However, the responses demonstrated in our series of experiments were quite similar. Shoot growth expressed either on a tissue, shoot or area specific basis decreased over time during both the summer and fall as either ambient temperatures increased (summer) or decreased (fall) from a temperature range (15–17°C) where maximal growth has been observed in the field (Evans et al., 1986; Moore et al., 1996). New leaf production decreased dramatically during the course of the summer experiment with average leaf production intervals (PI) exceeding 60 days in the high light treatments and 80 days in the low light treatments. Shoot growth increased over time during the spring, especially in medium and high light treatments (Fig. 2). The responses to light treatment were consistent throughout all three experiments with lowest growth rates observed in the low light treatments (Table 5; Fig. 2). During the spring and summer higher growth rates were observed in the medium light treatments than either the high shade treatments with low water column transmittance, or the high light treatments with corresponding high epiphyte loads (Table 5; Fig. 2). Water column

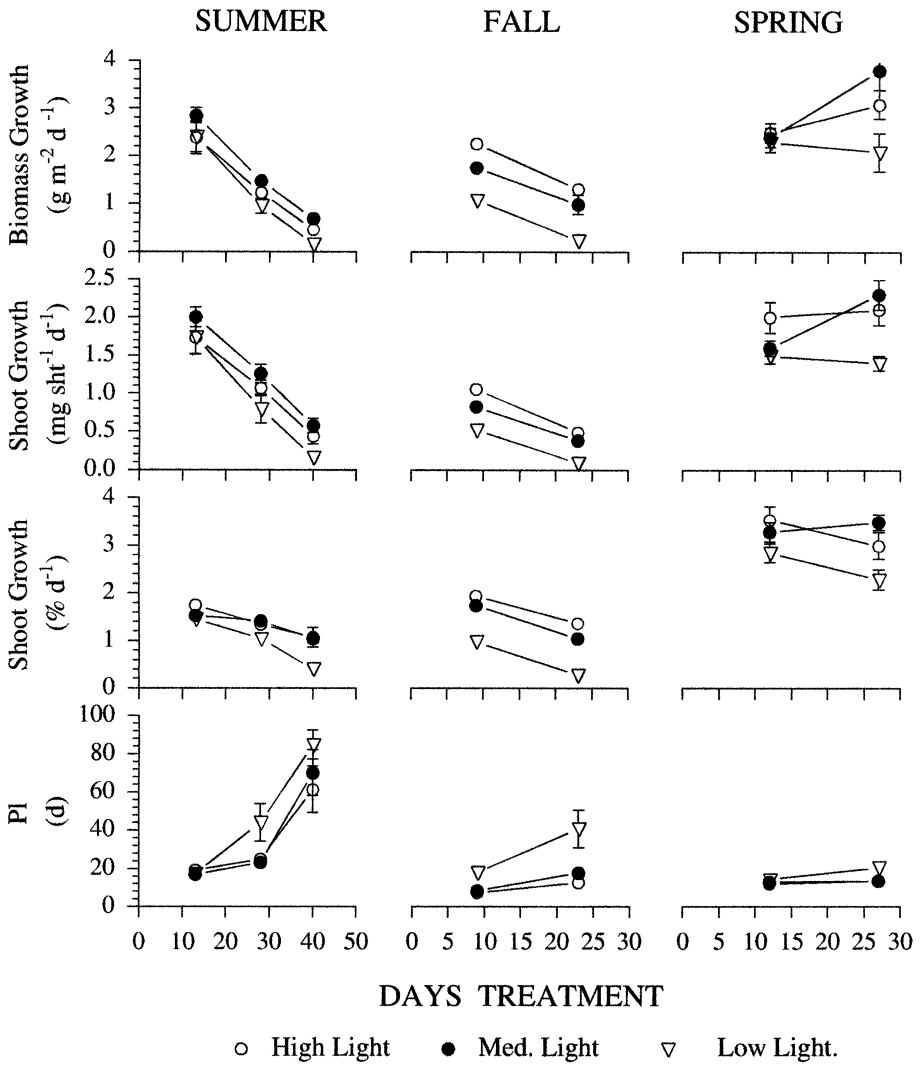

Fig. 2. *Zostera marina* growth responses to treatments. Light level by Time. Mean±SE. PI, plastochrone interval.

nutrient enrichment was associated with significantly lower ($P < 0.05$) eelgrass growth only during the summer and spring (Table 4) and then only at the highest light levels, with no measurable effects during the fall (Table 5; Fig. 2).

Z. marina weight paralleled the patterns observed with growth measurements: decreasing mass over time during the summer and fall, with significant decreases in both shoot and root–rhizome mass associated with decreasing light levels (Tables 5 and 6; Fig. 4). Nutrient additions resulted in significantly lower shoot and root–rhizome mass during the spring (Table 6). These differences were most pronounced after 4 to 6 weeks

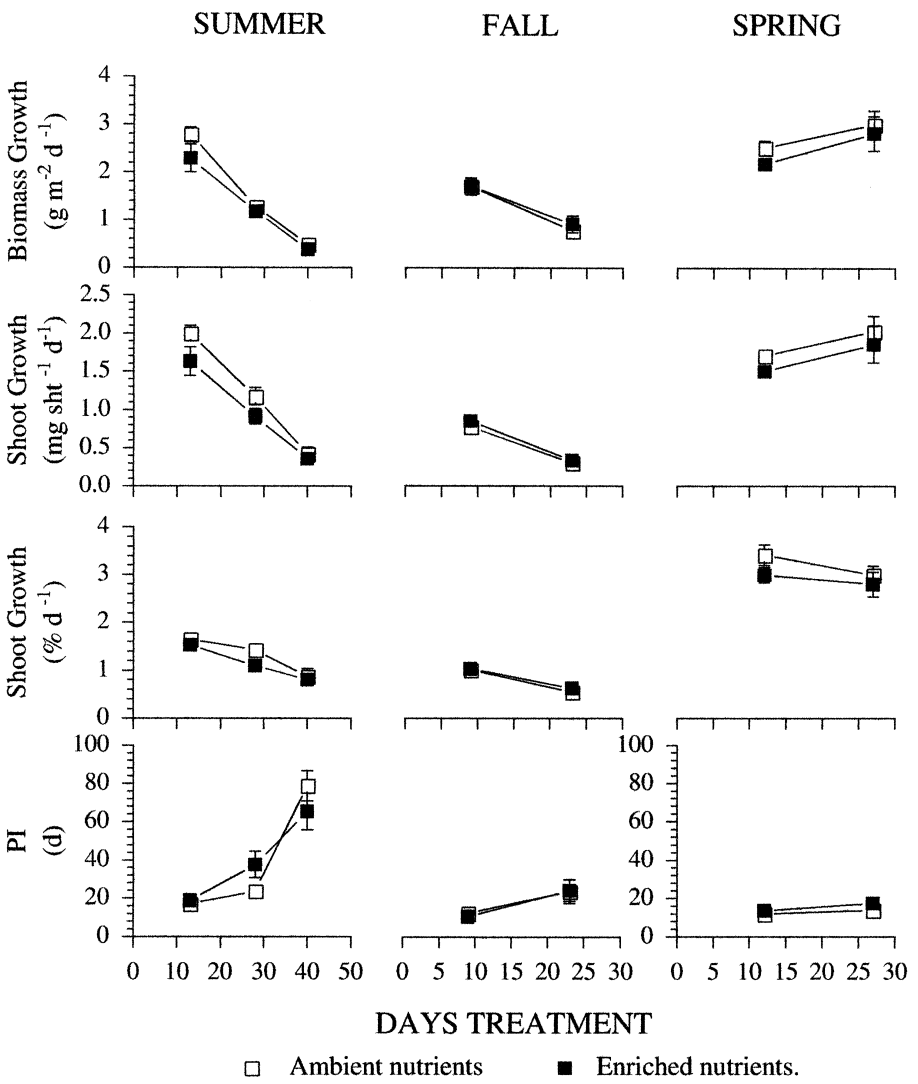

Fig. 3. *Zostera marina* growth responses to treatments. Nutrient level by Time. Mean±SE. PI, plastochrone interval.

of treatment (Fig. 4) and then only at the medium and high light levels (Table 5). By the end of the spring experiment shoot mass had decreased in the nutrient enriched compared to ambient nutrient, high and medium light treatments as leaf loss of the heavily epiphytized older leaves increased. Shoot mass was also significantly lower ($P < 0.05$) in enriched treatments during the summer (Tables 5 and 6) reflecting the lower specific growth rate observed with enrichment. Root–rhizome mass was unaffected by enrichment however (Tables 5 and 6; Fig. 5).

Table 5
Mean (SE) *Z. marina* growth, shoot and root–rhizome (R–R) mass and epiphyte mass at the end of each seasonal study

Season	Treatment		Shoot growth (mg shoot^{-1} d^{-1})	Shoot mass (g shoot^{-1})	R–R mass (g shoot^{-1})	Total epiphyte mass (g g^{-1})
	Nutrients	Light				
Summer	Ambient	High	0.45 (0.19)	0.24 (0.06)	0.33 (0.07)	4.66 (0.58)
	Ambient	Medium	0.64 (0.06)	0.33 (0.04)	0.39 (0.04)	2.04 (0.26)
	Ambient	Low	0.16 (0.08)	0.07 (0.07)	0.16 (0.07)	2.39 (0.28)
	Enriched	High	0.42 (0.08)	0.17 (0.07)	0.29 (0.10)	2.61 (0.24)
	Enriched	Medium	0.52 (0.17)	0.24 (0.13)	0.32 (0.13)	2.01 (0.23)
	Enriched	Low	0.15 (0.06)	0.04 (0.01)	0.10 (0.03)	2.37 (0.28)
Fall	Ambient	High	0.44 (0.23)	0.50 (0.02)	0.62 (0.05)	4.18 (1.04)
	Ambient	Medium	0.41 (0.65)	0.36 (0.07)	0.49 (0.07)	1.53 (0.18)
	Ambient	Low	0.07 (0.02)	0.11 (0.03)	0.22 (0.02)	0.91 (0.06)
	Enriched	High	0.54 (0.09)	0.27 (0.08)	0.60 (0.07)	4.62 (1.55)
	Enriched	Medium	0.36 (0.08)	0.23 (0.07)	0.54 (0.04)	2.52 (0.40)
	Enriched	Low	0.10 (0.05)	0.14 (0.04)	0.29 (0.07)	1.44 (0.18)
Spring	Ambient	High	2.37 (0.71)	1.28 (0.47)	0.64 (0.21)	1.50 (0.50)
	Ambient	Medium	2.39 (0.17)	1.33 (0.11)	0.67 (0.08)	1.64 (0.56)
	Ambient	Low	1.34 (0.49)	0.72 (0.14)	0.30 (0.04)	1.18 (0.32)
	Enriched	High	1.78 (0.65)	0.96 (0.19)	0.42 (0.12)	17.5 (4.10)
	Enriched	Medium	1.87 (0.95)	1.08 (0.40)	0.43 (0.15)	2.93 (0.40)
	Enriched	Low	1.34 (0.58)	0.75 (0.28)	0.31 (0.09)	1.02 (0.14)

3.3. Epiphyte response

Epiphyte responses to the effects of light and time were generally consistent among seasons as either microepiphyte or macroepiphytes mass increased significantly with time and light level (Tables 5 and 6; Fig. 4). During the summer experiment microepiphyte and macroepiphyte responses to treatments were similar, measured on both the natural, seagrass leaf blade as well as the artificial eelgrass substrates. Epiphyte accumulation on the artificial eelgrass demonstrated no significant effect of nutrient enrichment ($P > 0.10$), however as with the seagrass epiphytes, the effects of light and time were significant ($P < 0.05$). Both macroepiphytes and microepiphyte accumulation rates were similar and directly related to increasing water column light availability when expressed on a dry mass basis (Fig. 6). The higher organic content of microepiphytes compared to macroepiphytes (18% vs. 14%) resulted in greater microepiphyte accumulation rates when determined on an organic mass basis (Fig. 6).

During the fall and spring the microepiphytes and macroepiphytes responded differently to treatment effects. During the fall macroepiphytes mass remained very low while microepiphyte mass increased with increasing light availability (Fig. 5) resulting in epiphyte loads approximately four times shoot mass at the highest light levels (Table 5). Microepiphytes in enriched treatments were higher by the end of the experiment

Table 6
Analysis of variance of *Zostera marina* and attached epiphyte responses with repeated measurements on microcosms. N, nutrient effect; L, light effect; T, time effect. Bold indicates significant effect ($P < 0.05$)

Treatment	DF	Shoot mass (g shoot^{-1})		R–R mass (g shoot^{-1})		Microepiphyte (g g^{-1})		Macroepiphyte (g g^{-1})	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Summer									
N	1	6.30	**0.015**	0.70	0.408	0.84	0.363	6.70	**0.013**
L	2	6.52	**0.003**	6.52	**0.003**	14.00	**0.000**	9.49	**0.000**
N×L	2	0.52	0.592	1.49	0.236	0.23	0.793	2.63	0.082
T	2	86.19	**0.000**	35.34	**0.000**	9.90	**0.000**	4.68	**0.014**
N×T	2	3.14	0.052	1.24	0.298	0.80	0.454	0.95	0.394
L×T	4	0.34	0.850	1.07	0.380	1.92	0.122	2.35	0.066
N×L×T	4	1.62	0.180	2.20	0.082	0.69	0.602	1.11	0.358
Fall									
N	1	1.09	0.303	0.08	0.783	1.16	0.290	0.46	0.501
L	2	30.84	**0.000**	10.19	**0.000**	13.39	**0.000**	6.08	**0.006**
N×L	2	0.20	0.816	1.58	0.221	0.07	0.930	1.30	0.287
T	2	107.57	**0.000**	7.48	**0.010**	23.55	**0.000**	30.08	**0.000**
N×T	2	0.02	0.880	0.50	0.482	0.88	0.356	0.36	0.552
L×T	4	0.24	0.786	5.46	**0.009**	5.17	**0.011**	5.76	**0.007**
N×L×T	4	0.35	0.707	1.34	0.276	0.06	0.938	1.13	0.334
Spring									
N	1	42.41	**0.000**	39.32	**0.000**	1.01	0.321	18.86	**0.000**
L	2	7.07	**0.002**	20.66	**0.000**	2.37	0.103	3.15	**0.050**
N×L	2	5.12	**0.011**	6.02	**0.002**	2.64	0.081	3.12	0.053
T	2	0.96	0.391	2.21	0.123	26.98	**0.000**	3.85	**0.028**
N×T	2	14.77	**0.000**	6.73	**0.003**	6.04	**0.004**	1.88	0.162
L×T	4	1.32	0.280	2.00	0.113	4.19	**0.002**	1.45	0.230
N×L×T	4	1.79	0.151	1.81	0.147	1.29	0.285	2.25	0.080

when compared to ambient treatments, while no such trend was observed with the macroepiphytes (Fig. 5 and Table 5). Conversely, in the spring significant treatment effects of nutrient enrichment and increased water column light availability on macroepiphytes were observed, with no concomitant microepiphyte response. Macroepiphytes/seagrass shoot ratios exceeded 10:1 and were greatest in the high light, enriched treatments after 4 weeks of exposure (Figs. 4 and 5). There was no significant effect of nutrient enrichment on macroepiphyte response under low light conditions. This macroepiphyte assemblage consisted of fine strands of filamentous, tube dwelling diatoms, as well as the macroalga *Enteromorpha* sp. By the end of the experiment *Enteromorpha* formed dense mats that extended to the water surface and surrounded all but the youngest eelgrass leaves.

3.4. Nutrient uptake and release

The net exchange of inorganic nitrogen through the microcosms, calculated as the inflow minus the outflow, could be directly related to the combined mass of epiphytes

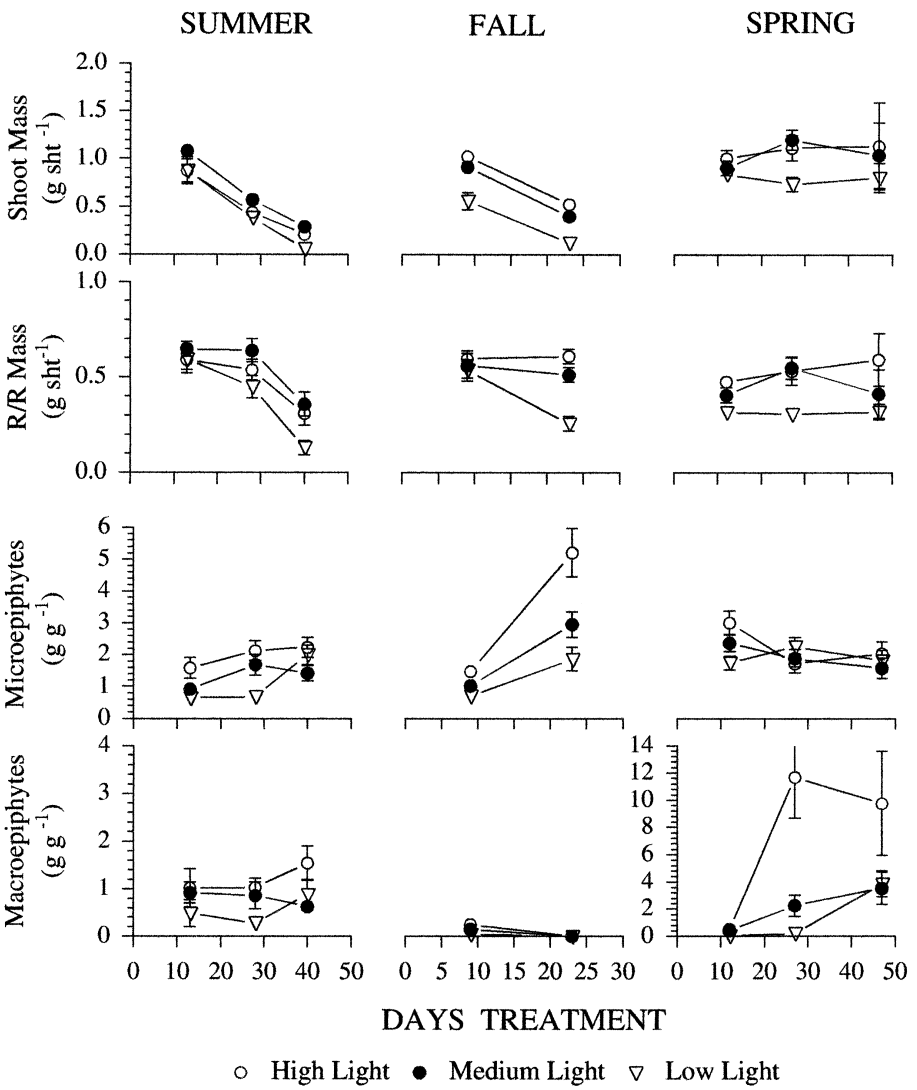

Fig. 4. *Zostera marina* and attached epiphyte responses to treatments. Light level by Time. Mean±SE.

and macrophytes in the microcosms (Fig. 7). Inorganic nitrogen uptake per unit biomass was higher during the summer than the spring and fall, and higher in the enriched treatments during the summer than the ambient treatments. In the low light treatments of all three experiments outflow of inorganic nutrients exceeded inflow with greatest net release of inorganic nitrogen apparent in the low light, ambient nutrient treatments.

The net exchanges of the microcosms for ammonium (NH_4^+), nitrate+nitrite (NO_x^-) and inorganic phosphate (PO_4^{3-}) are presented in Fig. 8. Nitrate (NO_3^-) comprised on average greater than 80% of the NO_x^- at all times and usually greater than 90% in the

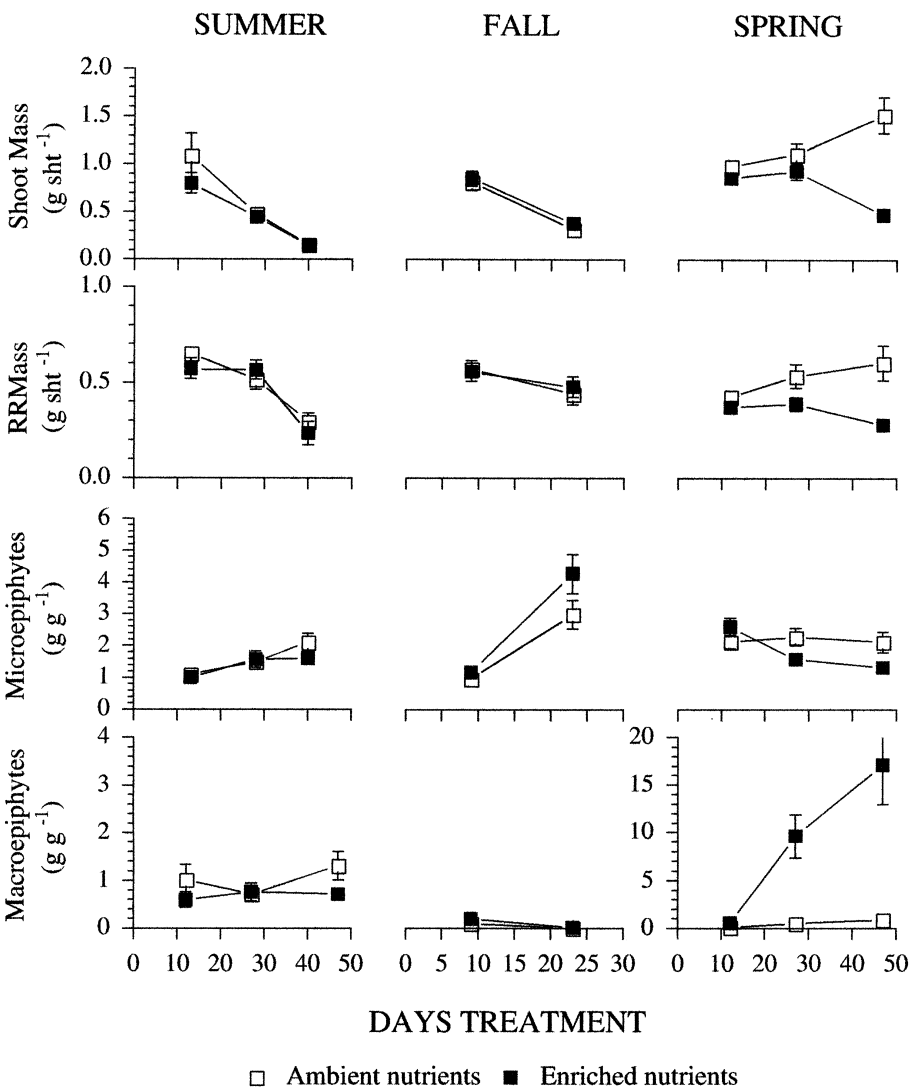

Fig. 5. *Zostera marina* and attached epiphyte responses to treatments. Nutrient level by Time. Mean±SE.

enriched treatments. During the summer under ambient nutrient levels, uptake of NO_x^- was markedly higher than NH_4^+ under medium and high light levels. However, under enriched conditions NH_4^+ uptake exceeded NO_x^- uptake even though mean NH_4^+ and NO_x^- inflow concentrations were similar. Although NO_x^- uptake was similar under ambient and enriched conditions during the summer (Fig. 8), mass (seagrass + macroepiphytes and microepiphytes) specific rates (Fig. 7) were greater with enrichment. During the fall experiment uptake of NH_4^+ and NO_x^-, especially under enriched conditions, were less than during the spring and summer, with NH_4^+ uptake again higher

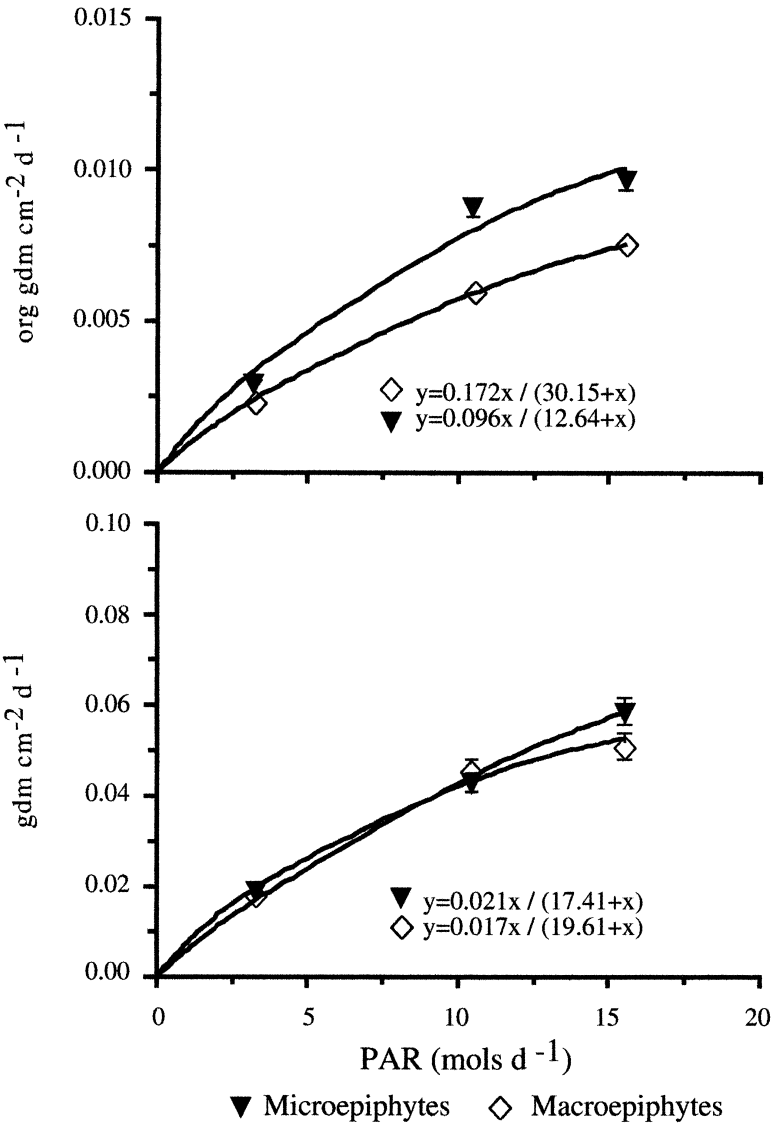

Fig. 6. Epiphyte accumulation rates on artificial seagrass. Summer experiment. Mean±SE.

than NO_x^- in the medium and high light, enriched treatments (Fig. 8). Extremely high levels of macroepiphyte mass were associated with high rates of NH_4^+ uptake from the medium and high light enriched chambers during the spring (Fig. 7). However, mass specific rates were comparable to the fall and lower than during the summer (Fig. 7). Inorganic phosphate demonstrated net export under all treatment combinations during the fall with net flux directly related to light level (Fig. 8). During the spring and summer PO_4^{3-} net uptake was observed only in the high light treatments with reduced

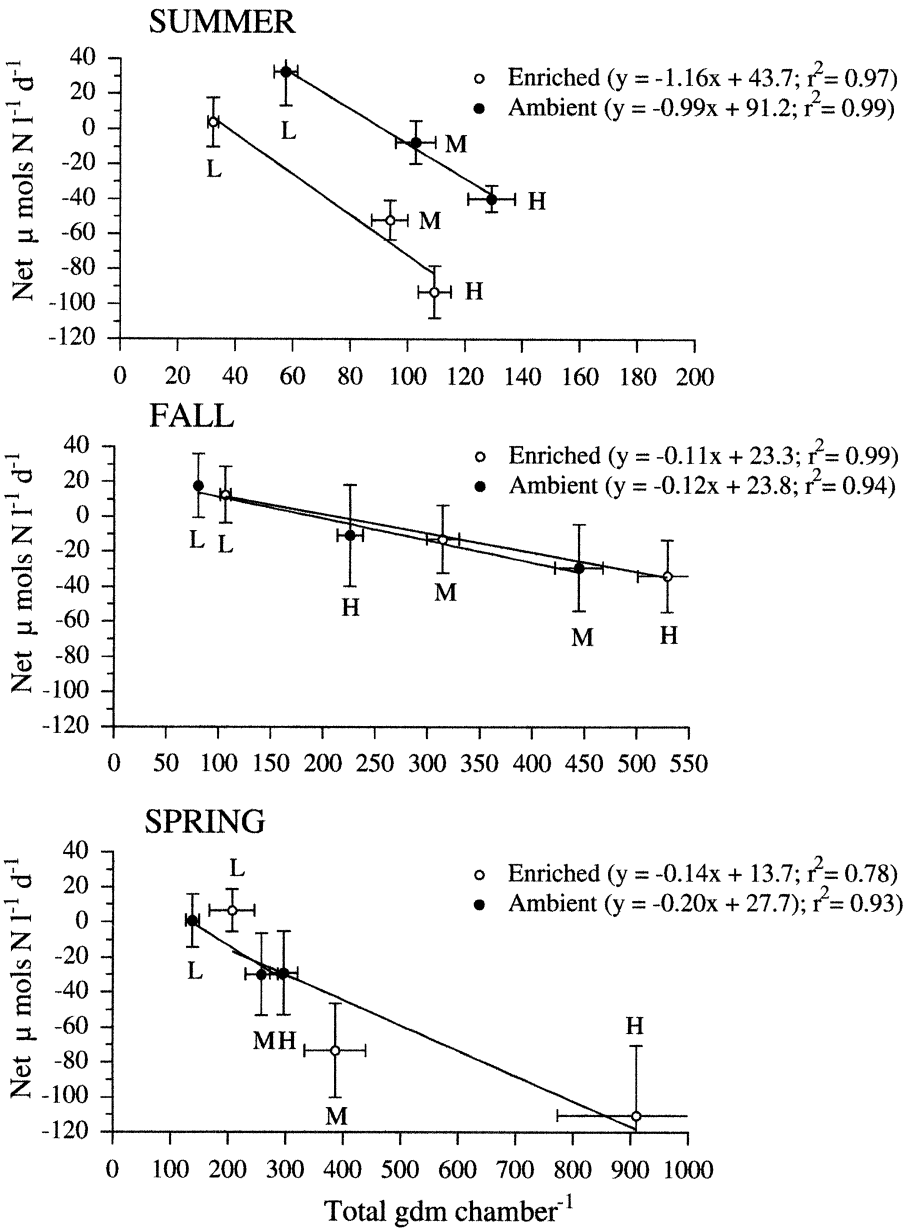

Fig. 7. Regressions between net uptake (−) or release (+) of inorganic N and dry mass of seagrass + epiphyte material. Data pooled from all treatments with mean ± SE. L, low light; M, medium light; H, high light.

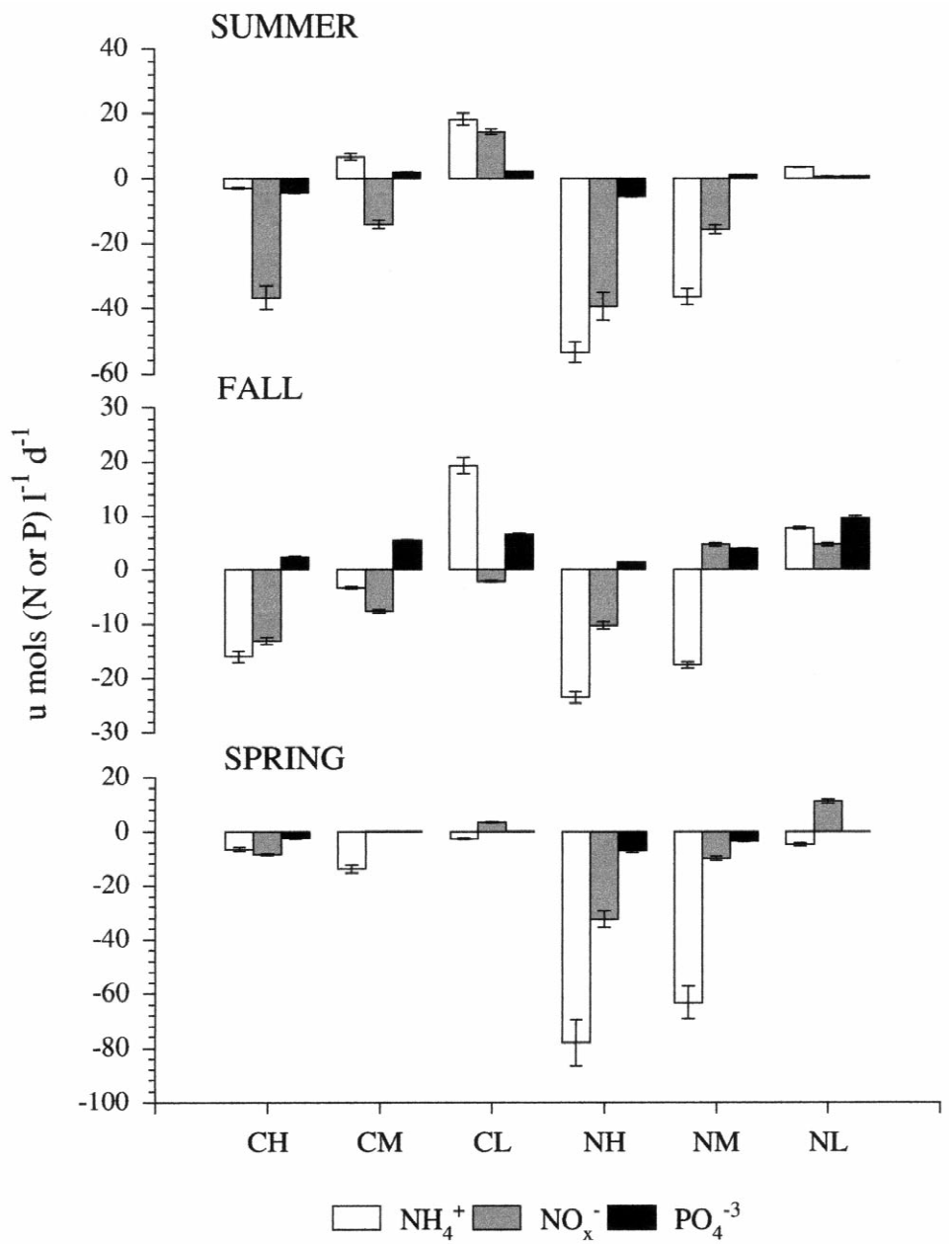

Fig. 8. Net uptake (−) or release (+) of inorganic N and P. Data pooled from all sampling periods with mean±SE. C, ambient nutrients; N, enriched nutrients; H, high light; M, medium light; L, low light.

uptake or release under medium and low light levels. As with inorganic nitrogen, mass specific rates of inorganic phosphate uptake were higher during the summer than during the fall or spring.

4. Discussion

4.1. Nutrient enrichment–light interactions

The results from this series of microcosm studies reveal that the relationships between light availability, nutrient enrichment, epiphyte accrual, and *Z. marina* response can vary seasonally, but water column light availability clearly governs seagrass response. In moderately, turbid (PAR Kd$>$1 m^{-1}) environments such as the York River estuary, given adequate grazer densities, observed levels of nutrient enrichment are unlikely to cause excessive epiphyte loads in areas where seagrasses have declined and transplants have been unsuccessful (Moore et al., 1996). Water column nutrient enrichment in this study resulted in increased epiphytic mass with concomitant decrease in *Z. marina* mass and production during the spring and only in treatments where water column light levels were high (42% of surface PAR). When light availability to the seagrass macrophytes and their attached epiphytes was reduced through microcosm shading (28% and 9% of surface PAR), the mass of both the seagrass and the epiphytes decreased. Presumably at these lower light levels, epiphyte growth was reduced and grazer population densities were sufficient to keep epiphytes at low levels. Therefore, the nutrient additions had no effect on epiphyte accrual or seagrass growth. During the summer and fall the 2$\times$ to 3$\times$ increases in ambient nutrient concentrations were insufficient to affect the accrual of epiphytes on the shoots or *Z. marina* growth at any of the light levels.

Most experimental studies which have previously observed relationships between water column nutrient enrichment, epiphytic growth and decreased seagrass production have been conducted under high light conditions (Burkholder et al., 1992; Neckles et al., 1993). In one other study where water column nutrient loadings and light conditions were simultaneously varied (Short et al., 1995) increased epiphyte mass and associated seagrass decline was evident in enriched treatments only when light levels exceeded 40% of incident irradiance. Twilley et al. (1985) conducted nutrient enrichment experiments in unshaded shallow ponds with *Potamogeton perfoliatus* and *Ruppia maritima*. They found that both epiphyte loads and phytoplankton induced turbidity increased with enrichment. As light availability at the *P. perfoliatus* canopy decreased from approximately 60 to 22% of surface irradiance due to algal blooms, epiphyte loads also increased from 0.67 to 2.11 gdm gdm^{-1} with enrichment, in contrast to the limited response observed here. One possible explanation is that epiphyte grazer populations in their ponds were low due to predation or other factors, and therefore epiphyte mass was able to increase in response to increased nutrient availability, even under reduced light levels. Neundorfer and Kemp (1993) similarly evaluated the effects of enrichment of water column inorganic nitrogen (N) and phosphorus (P) on seagrass–epiphyte relations using unshaded microcosms. Under highest levels of N+P enrichment (38 μmol N l^{-1} d^{-1} and 19 μmol P l^{-1} d^{-1}) epiphytes on *P. perfoliatus* increased even as phytoplankton

induced shading decreased light from 66 to 49% of ambient. Here again, epiphyte grazers were excluded from the experimental chambers. Taylor et al. (1995) and Lin et al. (1996) also studied the effects of nutrient enrichment on eelgrass communities in experimental mesocosms. In their experimental systems, which included invertebrate grazers as well as fish, they found epiphytes increased only in the mesocosms where the water column light levels remained high. They observed a decrease in eelgrass biomass in response to nutrient (N+P) enrichment, but only in individual mesocosms where phytoplankton blooms occurred and light attenuation was high ($Kd > 2\ m^{-1}$). In addition, they observed a marked decrease in epiphytes in response to nutrient additions, which they attributed to the decreased availability of light as a result of sustained phytoplankton blooms.

Neckles et al. (1993) concluded that at the 2× to 3× level of enrichment observed among currently and formerly vegetated sites in the lower Chesapeake Bay, the presence of grazers can be more important than water column nutrient supply in determining epiphyte biomass and decreased seagrass growth. The results of our experiments support this observation, although it may be further concluded that water column light availability is an additional factor which can affect nutrient–epiphyte–grazer interactions. This compounding light factor can be very important in systems such as the York River, where spring runoff brings not only increased nutrient loads but higher light attenuation related to increased suspended inorganic particle loads (Batuik et al., 1992; Moore et al., 1996, 1997). The accrual of epiphytes under low light conditions without grazers was not tested here. However, any factor which affects the level of grazer populations on *Z. marina* such as mortality or predation can have important implications for seagrass survival or successful recruitment into formerly vegetated areas.

4.2. Seasonal responses

Seasonal differences in the response of the seagrass microcosms to nutrient enrichment are related to a number of factors including temperature, light, water column nutrients, and grazing intensity. Decreasing temperatures combined with lowest seasonal incident light intensities during the fall experiment possibly limited the response of epiphyte communities to nutrient enrichment given the constant grazer densities applied here. During the spring, under conditions of moderate but increasing water temperatures and high seasonal light levels, the grazers capacity to control epiphytes was exceeded with enrichment. During the summer under conditions of high water temperatures and high light levels no epiphyte response to enrichment was observed. This suggests that elevated grazer metabolic and ingestion rates at this time may have increased their effective grazing rate on the epiphytes (Newell, 1970).

Decreases in seagrass mass and growth during the spring correlated with increased macroepiphytes abundance while microepiphytes remained low (Table 5). Excessive growth of macroepiphytes has been observed elsewhere (Harlin and Thorne-Miller, 1981; Cattaneo, 1983; Neckles et al., 1993; Valiela et al., 1997) and their negative affect on seagrass growth in microcosm experiments has been previously reported (Short et al., 1995). The inverse relationship between macroepiphyte and microepiphyte abundance observed here during the spring and fall suggests that their growth can be mutually

exclusive and the dominance of one or the other may depend on a number of, as yet, undetermined factors. For example, Short et al. (1995) found that in three replicate enriched mesocosms one became dominated with microepiphytes, one with macroepiphytes and one with phytoplankton. The factors which lead to the growth of one at the expense of the others was unknown but may have been related to not only the propagule supply, but the preferences of the grazer community, and controls on the grazers by predators (Lin et al., 1996). Regardless of which algal component developed, the negative effects on the seagrass were similar as the algae dominated at the expense of the seagrass. In our study, the dominance of macroepiphytes may have been related to the use of only snails as a grazer control. These grazers have been shown to be very effective in controlling diatom abundances (van Montfrans et al., 1982), however their effects on macroepiphytes are unknown. Neckles et al. (1993) used a more diverse grazer population and had less growth of macroepiphytes in their microcosms. This illustrates and supports the conclusion of others of the potential importance of the grazer populations and the controls on them in seagrass community dynamics including higher level predation (Wetzel and Neckles, 1986).

4.3. Nutrient uptake and nitrate stress

Although nutrient enrichment can affect seagrass growth by shifting community dominance to algal competitors, water column nitrate enrichment may cause negative, physiological effects on eelgrass unrelated to algal light attenuation (Burkholder et al., 1992, 1994). However, the obvious symptoms of direct nitrate toxicity (e.g., bright green color and weakened tissue structure, especially in the region of the meristem) as reported by Burkholder et al. (1992) were not observed here. Similarly, other recent enrichment studies of eelgrass have not reported such symptoms (Neckles et al., 1993; Short et al., 1995). One important difference among the responses may be related to the method of enrichment. Burkholder and her colleagues enriched their microcosm chambers once a day with a large pulse of nutrients with limited turnover of water (0.05 to 0.1 d^{-1}). In the other three studies (including this one), nutrient enrichment was more continuous and the turnover time of water in the chambers was much greater (2 to 16 d^{-1}). Active recycling of inorganic nitrogen within the chambers may also impact the effective dosages to the plants. For example, under highest enrichment levels of 35.0 μM NO_3^- N d^{-1}, Burkholder et al. (1992) observed that NO_3^- was maintained at approximately 200 to 300 μM throughout the course of one study. For temperate systems such as in Chesapeake Bay, we do not observe this level of enrichment (Moore et al., 1996; Sin et al., 1999).

Competition for nutrients with other algal and microbial components in the experimental chambers may also affect the direct impacts of nutrient additions on *Z. marina*. Using the measured eelgrass leaf and root–rhizome tissue nitrogen levels and the measured growth rates and biomass levels reported here (Tables 3 and 5), the total nitrogen requirements for the eelgrass were computed. These N requirements are exclusive of root uptake and internal plant recycling which may exceed 50% of plant needs (Borum et al., 1989; Pedersen and Borum, 1992). In the high light chambers during the summer, fall and spring, only 2 and 1%, 10 and 9%, and 9 and 2% of the

measured mean microcosm nitrogen uptake would be accounted for by *Z. marina* growth in the ambient and enriched treatments, respectively.

In spite of the apparent low proportion of total microcosm DIN uptake by the seagrass plants observed here during all seasons, the leaf tissue N concentrations increased with enrichment during both the summer and the spring at all light levels (Table 3). Increased tissue N with enrichment has been reported previously (e.g., Borum et al., 1989). This increase may reflect elevated tissue N levels that could be potentially toxic to the plants and may be inhibiting growth (Burkholder et al., 1992, 1994), or may be a result of luxury uptake (Short and McRoy, 1984; Borum et al., 1989) with no negative effect on growth. If toxicity were a significant factor here then there should have been a strong enrichment effect independent of light level. This was not generally observed. During the spring, decreased eelgrass growth response to enrichment was greatest at high light levels when tissue N concentrations were lowest but epiphyte levels were highest. During the summer there was decreased *Z. marina* growth with enrichment after 2 weeks of exposure (Fig. 5). At 4 to 6 weeks the enrichment effects on seagrass growth were not significant, although tissue N levels increased. Lack of enrichment effect on leaf tissue N levels during the fall correlated with highest seasonal tissue N, lowest water temperatures and solar irradiance, and highest seasonal water column nitrogen levels. This suggests that these factors can set limits on water column nitrogen uptake and subsequent storage.

Tissue N levels (Table 3) were within the range of levels reported for other eelgrass populations (Harrison and Mann, 1975; Thayer et al., 1977). In addition, increased tissue N concentration with long term shading has been observed elsewhere as growth requirements decrease (van Lent et al., 1995). Similarly, Buzzelli (1991) reported that eelgrass seasonal tissue N levels from plants in the lower York River were highest in the late fall and winter, and annually were inversely related to growth requirements.

Burkholder et al. (1992) have suggested that chronic nitrate enrichment may cause eelgrass decline by forcing the plants into carbon limitation, phosphorus limitation, or other severe internal nutrient imbalance, however little supporting evidence was found here. No changes in tissue carbon levels were observed in response to treatments. Although tissue N levels here (2–4% of dry wt. for shoots and 1–2% for root–rhizomes) were as high or higher than levels reported by Burkholder et al. (1992), tissue C levels were also higher (36–38% of dry wt. for shoots and 31–34% for root–rhizomes), suggesting little carbon limitation. Additionally, tissue N/P ratios consistently demonstrated no response to enrichment, indicating little potential for phosphorus limitation which could be related to eelgrass response.

5. Conclusions

Changing environmental conditions affect submersed seagrass communities through complex interactions involving many biotic and abiotic factors. While the effects of eutrophication on seagrasses have been well documented, seagrass response to anthropogenic nutrient enrichment can be mediated by both the density of the epiphytic grazer community as well as the degree of water column turbidity. Given normal grazer

densities and high turbidity, conditions found in the York River estuary in the lower Chesapeake Bay, the response of epiphyte communities is limited at moderate levels of water column nutrients (15–30 μM DIN and 2–3 μM DIP) which are characteristic of sites which no longer support seagrass. However, any factor which changes this balance, such as reductions in grazer populations, may have important implications for *Z. marina* survival. In addition, although direct toxicity due to nitrate enrichment may impact *Z. marina* populations under conditions of high pulsed inputs and limited turnover of water, in the conditions studied here where water exchange was high and nitrate input was constant but lower, toxicity was less apparent. This suggests that in some situations it may be the peak and not necessarily the long term average concentrations of nutrients which determine the stress level to *Z. marina* growth. Studies of the frequency, duration and intensity of changing habitat conditions as well as the complex interactions of the many biotic and abiotic factors which mitigate or enhance their effects of deteriorating habitat quality on seagrass growth remain principal to understanding man's role in the decline of seagrass communities worldwide.

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